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Original Communication

Hemoglobin F in sudden infant death syndrome: A San Diego SIDS/SUDC Research Project report

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Abstract

Whether levels of fetal hemoglobin (HbF), a possible marker of antecedent hypoxemia, are increased in sudden infant death syndrome (SIDS) compared to controls is unresolved. Our aims are to: (1) Compare percent fetal hemoglobin (%HbF) levels in SIDS and control cases, and (2) compare our findings with those reported in previous studies. Using Triton-acid-urea gel electrophoresis and quantitative densitometry, %HbF was determined in whole blood specimens obtained at autopsy from SIDS and control cases accessioned into the San Diego SIDS/SUDC Research Project database. The SIDS and control cases were not different with respect to mean age, gender, gestational age, method of delivery, birth weight, or mean autopsy interval; %HbF levels in SIDS and control cases were not significantly different. Given that our results were obtained using optimal methods in well-defined SIDS and control cases, we concur with others that %HbF is not elevated in SIDS.

Keywords: SIDS; Fetal hemoglobin; Sudden infant death; Hypoxia

1. Introduction

Sudden infant death syndrome (SIDS) is currently defined as "the sudden and unexpected death of an infant under one year of age, with onset of the lethal episode apparently occurring during sleep, that remains unexplained after a thorough investigation including performance of a complete autopsy, and review of the circumstances of death and the clinical history".[1] Its cause(s) remains unknown, but a body of literature suggests that some of these infants may have experienced acute, if not chronic, hypoxia prior to death.[2–4] Persistent elevation of fetal hemoglobin (HbF) is one marker of antecedent hypoxia that has been investigated, with conflicting results in studies of infants dying of SIDS. In their initial study in 1982, Zielke et al. found no difference in percent fetal hemoglobin (%HbF) in SIDS compared to control cases.[5] In their later investigation in 1989, Zielke et al. confirmed their earlier finding even when using three different methods of measurement.[6] However, subsequent studies yielded opposite findings, suggesting a delay in or faulty transition from HbF to adult hemoglobin (HbA) in SIDS.[7–9] Therefore, the aims of our study are to: (1) Compare %HbF in SIDS and control cases accessioned into the San Diego SIDS/SUDC Research Project database, and (2) compare our results with those reported in previous studies.

2. Methods

The Rady Children's Hospital and Health Center Institutional Review Board approved this study. The records of

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all infants who underwent autopsy at the San Diego County Medical Examiner's Office (ME) and were subsequently accessioned into the San Diego SIDS/SUDC Research Project (SDSSRP) database between 1993 and 2001 were searched for SIDS and control cases with available whole blood samples that had been collected during the autopsy in tubes containing sodium fluoride and maintained frozen at −70 °C before analysis. Samples from 142 infants were identified by number only before analysis of the %HbF of total hemoglobin at Florida Atlantic University in Boca Raton, Florida (GWP). All identifying, clinical, and autopsy data were maintained at the SDSSRP office in San Diego until the HbF analyses were completed.

For each case, the SDSSRP database contains information from two standardized protocols comprised of checklists regarding the death scene investigation and autopsy examination as well as the investigative and autopsy reports. Trained, experienced investigators from the ME are charged with obtaining this information within 30 h of death. The data are not complete for every case.

A diagnosis of SIDS was made in infants ($n = 77$) dying suddenly and unexpectedly when criteria fulfilling the San Diego definition were met.[1] Similarly, the diagnoses and causes of death in the non-SIDS control cases ($n = 30$) were based upon clinical, scene and autopsy investigation. None of our controls were known to have experienced intermittent or persistent hypoxemia.

The %HbF levels from autopsy whole-blood samples (stored for periods ranging from 1 to 9 years) were determined by previously described methods.[9] Briefly, using Triton-acid-urea gel electrophoresis and quantitative densitometry, the relative intensities of the separated globin chains were determined. The %HbF was determined by hemoglobin subunit analysis. The %HbF was calculated as the sums of the intensities of the γ-chains ($^{G}\gamma + {}^{A}\gamma$) divided by the sum of the intensities of the γ-chains plus β-chain. This technique is highly reproducible in whole blood.[9]

Table 1
Manner and cause of death for 107 cases of sudden infant death, 1993–2001

Manner	Cause of death	Number	Cases
Natural	SIDS		77
	Pneumonia	6	20
	Cardiac disorder	5	
	Aspiration	4	
	Sepsis	2	
	Seizure disorder, metabolic disorder, intestinal infarct	3	
Accident	Suffocation	5	5
USID[a]		5	5
Total			107

[a] Unexplained sudden infant death.[1]

3. Data analysis

SIDS and control cases were compared to one another with respect to demographic variables, birth history, and autopsy findings. Categorical variables were analyzed using the chi-square test or Fisher's exact test, along with odds ratios. Continuous data were analyzed with two-sample *t*-tests and are summarized using Means ± Standard Deviations. The GLM (General Linear Model) Univariate procedure in SPSS was used to perform an analysis of covariance (ANCOVA) to determine if the variable (SIDS

Table 2
Comparison of SIDS and control cases

	SIDS ($n = 77$)	Controls ($n = 30$)	*P*
Age (days)			NS[a]
Range	19–323	3–272	
Mean ± SD	101.4 ± 60.4	95.4 ± 77.9	
Gender			NS
Male	48 (62%)	16 (53%)	
Female	29 (38%)	14 (47%)	
Race			NS
White non-Hispanic	39 (51%)	7 (23%)	
Black non-Hispanic	10 (13%)	7 (23%)	
Hispanic (any race)	20 (26%)	13 (43%)	
Asian/Pacific Islander	5 (6%)	2 (7%)	
Other	3 (4%)	1 (3%)	
Gestation	$n = 72$	$n = 26$	NS
Full term	54 (75%)	21 (81%)	
Pre-mature[b]	18 (25%)	5 (19%)	
Delivery	$n = 65$	$n = 26$	NS
Vaginal	46 (71%)	21 (81%)	
Cesarean	19 (29%)	5 (19%)	
Birthweight (g)	$n = 65$	$n = 26$	NS
Range	510–4224	1332–4734	
Mean ± SD	2992 ± 868	3189 ± 767	
Median	3090	3175	
Maternal age	$n = 61$	$n = 24$	NS
⩽16	2 (3%)	1 (4%)	
17–19	5 (8%)	0	
20–24	20 (33%)	9 (38%)	
25–29	16 (26%)	6 (25%)	
30–34	11 (18%)	8 (33%)	
⩾35	7 (11%)	0	
Pre-/post-natal smoke exposure	$n = 49$	$n = 15$	0.01
Yes	13 (27%)	10 (67%)	
No	36 (73%)	5 (33%)	
%HbF			NS
Range	8.9–86.1%	8.4–83.1%	
Mean ± SD	46.1 ± 22.4%	47.3 ± 24.8%	
Median	44.9%	49.8%	
PMI[c] Range (h)	4.9–33.5	4.9–34.3	
Mean PMI ± SD (h)	21.4 ± 6	20 ± 9.1	NS

[a] Not significant.
[b] Pre-maturity defined as <37 weeks gestation.
[c] PMI = Post-mortem interval.

versus control group) was significantly related to the dependent variable (%HbF) after the variation due to the covariate (post-conceptional age (PCA)) had been removed. Calculations were performed with SPSS Version 12.0. A *P*-value less than 0.05 was considered significant.

4. Results

The blood specimens of 75% (107) of the 142 cases were satisfactory for analysis. In addition to the SIDS cases, Table 1 lists cause of death for the 30 control cases. Table 2 compares demographic, birth and autopsy characteristics of the SIDS cases to the control cases. The disparate groups were statistically similar with regard to all findings except for exposure to cigarette smoke.

Table 3 provides the mean %HbF for SIDS by risk factors. Black non-Hispanic infants had the highest mean %HbF levels among all racial-ethnic groups; however, this difference was statistically significant only when compared to the "Other" racial-ethnic group, comprised of Asian/Pacific Islander, Native American/Alaskan Native and multiracial infants. For SIDS and controls combined, there was a statistically significant effect for term (pre-mature infants had a mean level of 62.1% HbF compared to full term infants with a mean of 43.2%, $p = 0.01$) but this effect was not significant within the SIDS group alone.

Fig. 1 shows the individual data points for %HbF by group for 30 control and 77 SIDS cases. %HbF values for both groups are plotted against PCA (as opposed to post-natal age) since the normal post-natal decline in HbF levels is developmentally programmed from conception rather than birth. After adjusting for PCA, %HbF levels for SIDS cases and controls did not differ significantly.

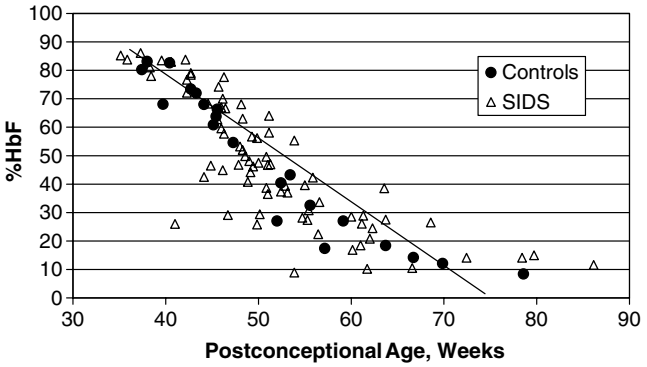

Fig. 1. Relationship of percent fetal hemoglobin (%HbF) and post-conceptual age in SIDS and control cases. The *R* values for SIDS and control cases are 0.66 and 0.87, respectively. The *R* value for the pooled samples is 0.726, indicating no difference between the two groups after accounting for PCA.

Table 3
Relationship of SIDS risk factors and %HbF

Risk factors	Fetal hemoglobin level (% ± SD)	*P*
Race/ethnicity ($n = 77$)		.03
White non-Hispanic (39)	44.1 ± 21.2	
Black non-Hispanic (10)	60.3 ± 23.2	
Hispanic, any race (20)	49.4 ± 23.5	
Other[a] (8)	30.0 ± 13.2	
Gestation ($n = 72$)		NS[b]
Full term (54)	41.6 ± 20.1	
Pre-mature[c] (18)	63.2 ± 21.6	
Maternal age ($n = 61$)		NS
⩽16 (2)	57.5 ± 7.8	
17–19 (5)	48.7 ± 21.9	
20–24 (20)	42.4 ± 22	
25–29 (16)	57.3 ± 21.4	
30–34 (11)	46.7 ± 23.9	
⩾35 (7)	51.6 ± 19.7	
Pre-/post-natal smoke exposure ($n = 49$)		NS
Yes (13)	48.8 ± 21.2	
No (36)	46.2 ± 23.2	

[a] Includes Asian/Pacific Islander, Native American/Alaskan Native and multiracial infants.
[b] Not significant.
[c] Pre-maturity is defined as <37 weeks gestation.

5. Discussion

Our study showed no statistical difference in %HbF between SIDS and control cases. Our data also revealed that our SIDS and control cases were not different with respect to mean age, gender, gestational age, method of delivery, birth weight, ethnicity, maternal age or mean post-mortem interval (Table 2). However, exposure to cigarette smoke, either pre- or post-natal, was significant at $p = 0.01$; more than twice the proportion of controls were exposed to cigarette smoke compared to the SIDS group. One explanation for this finding could be that we have responses to this question for only 64% of SIDS infants and 50% of control infants. Another possibility is that SIDS families may under-report a behavior known to increase the risk of SIDS.

The conflicting results of eight previous studies comparing HbF in SIDS and control cases are shown in Table 4.[5–12] Several possibilities may account for these differences, including inconsistent criteria to diagnose SIDS. Most of the previous studies do not indicate which SIDS definition was used, although it is a reasonable to assume that those published prior to 1991 (when the NICHD definition.[13] was published) would have used the original Beckwith definition.[14] The lack of scene investigation and reconstruction may lead to inaccurate ascertainment of cause of death through failure to identify sleep environments conducive to lethal rebreathing of expired gases or positional asphyxia. Diagnostic discrepancies could result from the lack of ancillary studies in cases for which a cause of death was not determined through gross and microscopic examination; diseases related to intoxication, electrolyte imbalance, or abnormal metabolism would not have been

Table 4
Comparison of studies evaluating fetal hemoglobin in SIDS

	Reference	Method[a]	SIDS	Living controls	Autopsy controls	Other	%HbF formula	Result	Critique
No difference	Zielke (1982)[5]	AD	85		15		Not specified	HbF not elevated in SIDS	Method has poor reproducibility and is not recommended on material with >40% HbF without dilution
	Kline (1989)[12]	HPLC	38	67	10		Not specified	HbF not elevated in SIDS	Measured native HbF; older, less reliable method
		RID	38	67	10				
	Zielke (1989)[6]	HPLC	67	80	22		$\frac{G\gamma+A\gamma+At\gamma}{all\ \gamma+\beta}$	HbF not elevated in SIDS	Did not use PCA[b]
		PGE	67	80	22				
		IF	67	80	22				
	Cheron (1989)[11]	AD	19	266			Betke[19] with Pembry[20] modification	No difference in total Hb or HbF	Did not use PCA[b], measured native HbF; used older, less reliable method
		CAE	19	266					
	Krous et al. (this article)	TGE	77		30		$\frac{G\gamma+A\gamma}{all\ \gamma+\beta}$	HbF not elevated in SIDS	Samples stored over nine year period
Difference	Giulian (1987)[10]	IEF	59	32	8		$\frac{A\gamma+G\gamma\ peaks}{\gamma+\beta\ peaks}$	HbF elevated in SIDS ($p < 0.025$); more pronounced at PCA > 50 weeks ($p < 0.0005$)	Small number of autopsy controls; samples stored over a five year period
	Fagan (1992)[7]	AD	135	570			$\frac{A\gamma+G\gamma\ peaks}{\gamma+\beta\ peaks}$	HbF elevated in full-term SIDS compared to full-term control group ($p < 0.001$)	SIDS infants include deaths from accidents and infection
	Gilbert-Barness (1993)[8]	HPLC	54	22	17	Normal values from literature	$\frac{G\gamma+A\gamma+At\gamma}{all\ \gamma+\beta+pre\beta}$	HbF elevated in SIDS compared to controls ($p = 0.015$) and literature ($p < 0.01$)	Combined living and autopsy controls into one group
	Perry (1997)[9]	TGE	47	17	33		$\frac{G\gamma+A\gamma}{all\ \gamma+\beta}$	HbF elevated in SIDS compared to controls ($p < 0.001$)	Samples collected from several locales; living and autopsy controls combined into one control group

CAE = citric agar electrophoresis; HPLC = High performance liquid chromatography; IEF = isoelectric focusing; IF = immunoflourescence microscopy; PGE = polyacrylamide-gel electrophoresis; RID = radial immunodiffusion; TGE = triton-acid-urea gel electrophoresis and quantitative densitometry.

[a] AD = alkali denaturation.

[b] PCA = Post-conceptual age.

identified. Absent this vital aspect of the investigation, infants dying of other causes may be included in the SIDS group, thereby increasing the variability of HbF in the study and control groups.[9,15]

Another possible explanation may lay in the use of inappropriate controls matched for post-natal age instead of post-conceptual age.[7,16] Further, the wide variation in methods to quantify HbF in previous studies may have contributed to the differing results (Table 4).

Our study has limitations. The number of controls (30) is relatively small compared to the number of SIDS cases (77). The available blood specimens were satisfactory for analysis in only 107 (75%) of the 142 identified cases. We are unable to determine if the inclusion of these other cases would have influenced the results. We cannot evaluate the potential effect of time the sample was frozen before analysis, although the method we used (HPLC) separates post-translational modifications including freezing and storage artifacts.[12]

Conversely, our study is particularly strengthened by consistent case evaluation through the application of the most current SIDS definition.[1] as well as use of standardized protocols for scene investigation and autopsy, including extensive ancillary testing.[17,18] We had 77 SIDS cases which were thoroughly examined, compared to other studies that generally had fewer cases, or less stringent ascertainment of cause of death. None of our 30 control cases had a known history of hypoxemia. In addition, we compared data from deceased infants, as opposed to using living controls or literature-based normal values. Finally, we calculated %HbF based on a method with proven reliability.

In conclusion, we concur with other investigators who found no differences in %HbF between SIDS and controls. Our findings are especially compelling because our SIDS cases fulfill the rigorous criteria of the 2004 San Diego SIDS definition.

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